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REVIEW

Sensing and reacting to microbes through the inflammasomes

Luigi Franchi, Raul Muñoz-Planillo & Gabriel Núñez

Inflammasomes are multiprotein complexes that activate caspase-1, which leads to maturation of the proinflammatory cytokines interleukin 1β (IL-1β) and IL-18 and the induction of pyroptosis. Members of the Nod-like receptor (NLR) family, including NLRP1, NLRP3 and NLRC4, and the cytosolic receptor AIM2 are critical components of inflammasomes and link microbial and endogenous danger signals to the activation of caspase-1. In response to microbial infection, activation of the inflammasomes contributes to host protection by inducing immune responses that limit microbial invasion, but deregulated activation of inflammasomes is associated with autoinflammatory syndromes and other pathologies. Thus, understanding inflammasome pathways may provide insight into the mechanisms of host defense against microbes and the development of inflammatory disorders.

The innate immune system is the first line of defense against microbial infection and is activated by the engagement of germline-encoded pattern-recognition receptors (PRRs) in response to microbes[1]. PRRs recognize the presence of unique microbial components called pathogen-associated molecular patterns or endogenous damage-associated molecular patterns generated in the setting of cellular injury or tissue damage[2]. In response to infection, PRR activation initiates signal-transduction pathways that ultimately culminate in host defense responses that eliminate microbial invasion. A major inflammatory pathway is activation of the inflammasome, a multiprotein platform that activates caspase-1 (ref. 3). Once activated, caspase-1 proteolytically cleaves the cytokine precursors pro-interleukin 1β (pro-IL-1β) and pro-IL-18; this is critical for release of the biologically active forms (L-1β and IL-18) and triggers proinflammatory and antimicrobial responses[4]. Active caspase-1 can also cleave less-defined protein substrates to regulate the induction of pyroptosis, autophagy and bacterial degradation through mechanisms that are poorly understood[4]. So far, four inflammasomes have been identified; these are named after the PRR that regulates their activity: NLRP1, NLRP3, NLRC4 and AIM2. Except for AIM2, these inflammasomes contain a PRR that belongs to the Nod-like receptor (NLR) family. NLRs are intracellular PRRs defined by a tripartite structure comprising the following: an amino-terminal caspase-recruitment domain (CARD), pyrin domain, acidic transactivating domain or baculovirus inhibitor repeat that mediates downstream protein-protein interactions; a central nucleotide-binding-and-oligomerization domain (Nod) that mediates self-oligomerization; and carboxy-terminal leucine-rich repeats (LRRs) that are thought to sense different microbial and endogenous damage stimuli[4]. In this review, we focus on the activation, regulation and function of NLR inflammasomes with an emphasis on their interaction with microbes and their role in host defense.

Department of Pathology and Comprehensive Cancer Center, University of Michigan, Ann Arbor, Michigan, USA. Correspondence should be addressed to G.N. (bclx@umich.edu).

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NLRC4 inflammasome: microbe recognition and activation

NLRC4 is important for the activation of caspase-1 in macrophages infected with pathogenic bacteria, including *Salmonella enterica* serovar Typhimurium (*S. enterica*)[5,6], *Legionella pneumophila*[7–9] and *Pseudomonas aeruginosa*[10,11]. The activation of caspase-1 by these pathogenic bacteria requires a functional bacterial secretion system that has been suggested as a link between bacterial pathogenicity and NLRC4 activation[3]. These secretion systems, which include the type III secretion system (T3SS) and type IV secretion system (T4SS), act as molecular needle-like structures that inject effector proteins into the cytosol of host cells and are critical for pathogen colonization. Flagellin, the main component of the flagellum, is important for activation of the NLRC4 inflammasome[5,6]. Because the delivery of purified flagellin to the macrophage cytosol triggers caspase-1 activation through NLRC4 (refs. 5,6), it had been thought that NLRC4 is activated in macrophages via the leakage of small amounts of flagellin through a T3SS (for example, *S. enterica* and *P. aeruginosa*) or T4SS (for example, *L. pneumophila*) during infection[12]. However, *Shigella flexneri*, an aflagellated pathogenic bacterium, also induces activation of the NLRC4 inflammasome through the T3SS[13]. Furthermore, flagellin-deficient *S. enterica* and *P. aeruginosa* can activate NLRC4 at high ratios of bacteria to macrophages, which further suggests that factors other than flagellin can induce activation of the NLRC4 inflammasome[14,15]. Initial insights into the flagellin-independent pathway were provided by the observation that proteins that form the basal body rod component of the T3SS, such as PrgJ, can activate the NLRC4 inflammasome. PrgJ-like proteins contain regions structurally homologous to the carboxy-terminal portion of flagellin[14], which is a critical portion of flagellin that is sufficient to trigger NLRC4 inflammasome activation[6,16]. However, the contribution of T3SS rod proteins to activation of the NLRC4 inflammasome is difficult to evaluate because bacterial pathogens

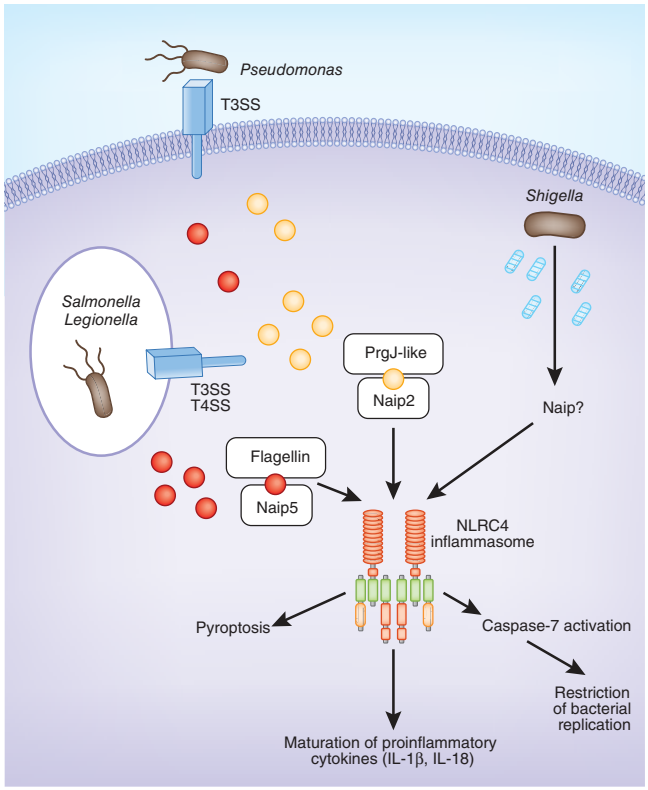

Figure 1 The NLRC4 inflammasome. Infection of macrophages with various Gram-negative bacteria, including *S. enterica*, *L. pneumophila* and *P. aeruginosa*, activates caspase-1 via NLRC4. A critical step is the cytosolic delivery of flagellin or PrgJ-like proteins through bacterial T3SS or T4SS. Flagellin is recognized by Naip5 or Naip6 (not shown here), whereas PrgJ-like proteins are recognized by Naip2. *S. flexneri* activates the NLRC4 inflammasome independently of flagellin through an unknown microbial product. Activation of caspase-1 via NLRC4 leads to the processing and release of IL-1β and IL-18, the processing of caspase-7 and the induction of other cellular activities that are poorly understood.

without a functional secretion system are impaired in the secretion of effector proteins and are therefore highly attenuated.

How does NLRC4 sense different structures such as flagellin and PrgJ-like proteins? Initial studies have shown a link between NLRC4 and Naip5 (neuronal apoptosis inhibitor protein 5), another member of the NLR family. Naip5 senses the carboxy-terminal region of flagellin and is required for the activation of NLRC4 in response to *L. pneumophila*[16]. In contrast, Naip5 is dispensable for NLRC4 activation in response to *S. enterica*, *P. aeruginosa* or flagellin purified from *S. enterica*[16]. Those puzzling results have been clarified by the observation that distinct Naip proteins link flagellin and PrgJ-like proteins to NLRC4. Whereas flagellin binds Naip5 and Naip6, PrgJ-like proteins interact with Naip2 (refs. 17,18). CprI, a subunit of the secretion system of *Chromobacterium violaceum*, binds to human NAIP[17]. Results obtained by reconstitution of the NLRC4 inflammasome in 293 human embryonic kidney cells also suggest that Naip proteins act upstream of NLRC4 to promote inflammasome activation[17,18]. Although the mechanism by which Naip proteins activate NLRC4 remains unclear, one model proposes that flagellin or PrgJ-like proteins bind to the LRRs of Naip proteins to induce a conformational change in the latter; this in turn induces the activation of NLRC4 (**Fig. 1**). A better understanding of the link between Naip proteins and NLRC4 is needed to explain why Naip5 is essential for the activation of caspase-1 in response to *L. pneumophila* but is dispensable in *S. enterica* infection despite the fact that flagellin from both pathogens binds to Naip5 (refs. 16–18). Because the amino terminus of flagellin can inhibit the interaction of flagellin with Naip5, the differences in the role of Naip5 in the recognition of *L. pneumophila* and *S. enterica* could depend on other factors that regulate the exposure or conformation of the carboxyl terminus, the portion of flagellin that is sufficient for NLRC4 activation[19]. Finally, *S. enterica* potently induces caspase-1 activation in human cells[20], but flagellin is not sensed by human NAIP[17], which raises questions about the molecular mechanism that induces caspase-1 activation in human cells.

Role of the NLRC4 inflammasome in host defense

The NLRC4 inflammasome regulates host defense by controlling the release of IL-1β and IL-18, bacterial degradation and pyroptosis. IL-1β and IL-18 have an important role in the host defense response to *S. flexneri*[21], but it is unknown whether the production of these cytokines depends on NLRC4 *in vivo*. In the case of *S. enterica* infection, caspase-1 and IL-18 both have a role in host defense[22,23]. NLRC4 does not have a role in host defense after orogastric infection in C57BL/6 mice[23,24], but it confers host protection to mice on the BALB/c background (lethal factor; G.N., unpublished observations). It was initially observed that activators of the NLRC4 inflammasome induce pyroptosis, a form of caspase-1-dependent cell death with features of both apoptosis and necrosis[25]. Notably, *S. enterica* represses expression of flagellin and T3SS during the systemic phase of infection, and downregulation of these factors prevents activation of the NLRC4 inflammasome[26]. In fact, mutants of *S. enterica*[26] or *L. monocytogenes*[27,28] that have enforced expression of flagellin cannot evade detection by the NLRC4 inflammasome and are highly attenuated. Furthermore, NLRC4 restricts *S. enterica* strains that overexpress flagellin independently of IL-1β and IL-18 by promoting the release of intracellular *S. enterica* from pyroptotic macrophages; these bacteria are consequently engulfed and killed by neighboring neutrophils[26]. However, the contribution of this protective mechanism to host defense during physiological infection with wild-type intracellular bacteria remains to be determined.

NLRC4 can also promote the degradation of pathogens inside macrophages. This is exemplified by studies of *L. pneumophila*, a Gram-negative intracellular bacterium that causes Legionnaires' disease, an acute form of pneumonia. After infection, *L. pneumophila* replicates in specialized vacuoles inside macrophages. Notably, NLRC4-dependent activation of caspase-1 restricts the intracellular growth of *L. pneumophila* at least in part by promoting the fusion of bacteria-containing vacuoles with lysosomes[9]. The ability to restrict *L. pneumophila* growth by NLRC4 requires expression of flagellin and host caspase-7, a proteolytic substrate of caspase-1, but not IL-1β and IL-18 (refs. 9,29). However, the mechanism by which caspase-7 promotes the fusion of *L. pneumophila*–containing vacuoles with lysosomes and bacterial degradation is unclear. Naip5 also has a critical role in restricting the growth of *L. pneumophila* inside macrophages; this can be partially explained by a role of Naip5 in linking cytosolic flagellin to NLRC4 activation[9,29,30]. However, analysis of A/J mice that express a mutant Naip5 protein that supports the replication of *L. pneumophila* and Naip5-null mice suggests that Naip5 may also act independently of NLRC4 to regulate *L. pneumophila* replication[31]. Although both mutant mice show greater bacterial replication than wild type do, the amino acids of Naip5 substituted in A/J mice are not important for flagellin binding or activation of the NLRC4 inflammasome[17], which suggests that Naip5 has a role in restricting bacterial replication that is distinct from its role in activating the

NLRC4 inflammasome. In addition to promoting bacterial degradation, NLRC4 and Naip5 can control *L. pneumophila* growth through the induction of pyroptosis, a phenotype evident under high multiplicity of infection[9,30]. Whereas macrophages deficient in Naip5, NLRC4, caspase-1 or caspase-7 show a notable phenotype in regulating *L. pneumophila* replication *in vitro*, the role of the flagellin-Naip5-NLRC4 signaling pathway in lung infection is more modest, which suggests that other signaling pathways can compensate for inflammasome deficiency *in vivo*[9,29,32].

The NLRP1 inflammasome

The initial description of the inflammasome was based on the assembly of the human NLRP1 inflammasome. Although the role of NLRP1 in immune responses remains poorly understood, its relevance is underscored by the association of variations of the gene encoding NLRP1 with generalized vitiligo, vitiligo-associated type I diabetes, Addison's disease, rheumatoid arthritis and Alzheimer's disease[3]. The domain structure of human NLRP1 comprises an amino-terminal pyrin domain, a centrally located Nod and LRRs, and carboxy-terminal FIINDs (function-to-find domains) and CARDs. Thus, NLRP1 differs from other NLRs proteins by having two signal-transduction domains; that is, a pyrin domain and CARD. Analysis of extracts of the THP-1 human myeloid cell line with macrophage-like properties has shown that NLRP1 forms a multiprotein complex containing the adaptor ASC, CARD8, caspase-5 and caspase-1 that has IL-1β-processing activity[33]. Subsequently, reconstitution of the NLRP1 inflammasome with purified components has shown that the minimal elements of the NLRP1 inflammasome are NLRP1, an NTP and caspase-1 (ref. 34). The activity of the reconstituted NLRP1 inflammasome is induced by muramyl dipeptide (MDP), and on the basis of those initial studies, caspase-1 has been proposed to be activated through a two-step mechanism: first, microbial MDP binds NLRP1 and changes its conformation, which allows it to bind an NTP; this in turn induces oligomerization of NLRP1 through its Nod, thus creating a platform for caspase-1 activation. The adaptor ASC is not essential for caspase-1 activation, probably because human NLRP1 can bind to caspase-1 directly through a CARD-CARD interaction, but the presence of ASC augments NLRP1-mediated caspase-1 activation[34]. Consistent with those results, macrophages stimulated with MDP in complex with titanium dioxide activate the NLRP1 inflammasome in an ASC-independent manner[35]. However, there is no direct evidence that MDP binds to NLRP1. Notably, NLRP1 and CARD8 are proteolytically processed, but it is still unclear whether these events are necessary for caspase-1 activation[36]. Thus, further work is needed to understand the activation of NLRP1 and the role of CARD8 and caspase-5 in the function of the NLRP1 inflammasome.

Unlike humans, who have a single *NLRP1* gene, mice have three tandem paralogs, *Nlrp1a*, *N1rp1b* and *Nlrp1c-ps* (also known as *Nalp1a*, *Nalp1b* and *Nalp1c*, respectively). Furthermore, different strain-specific alleles exist for *Nlrp1b*, and these genetic variants have led to the identification of NLRP1b as the sensor of *Bacillus anthracis* lethal toxin[37]. Lethal toxin, a metalloproteinase, is a bipartite toxin that consists of protective antigen and a pore-forming molecule that mediates the translocation of lethal factor into the host cytosol, where it activates caspase-1. Lethal toxin–induced caspase-1 activation, IL-1β production and pyroptosis require the susceptible *Nlrp1b* allele[37], whereas ASC is required for lethal toxin–induced production of IL-1β but is dispensable for pyroptosis[38]. *In vivo* experiments have shown that the product of *Nlrp1b* allelic variation has a protective role in *B. anthracis* infection and, consistent with a role for NLRP1b in activating the inflammasome, mice deficient in caspase-1 (*Casp1*$^{-/-}$) or IL-1β (*Il1b*$^{-/-}$) are more susceptible to *B. anthracis* infection[39]. Thus, the host-protective response mediated via the NLRP1B inflammasome depends on the production of IL-1β rather than the induction of pyroptosis. Although much progress has been made in elucidating the recognition of *B. anthracis* via the NLRP1B inflammasome, the mechanism by which lethal factor triggers activation of the NLRP1B inflammasome remains elusive.

The NLRP3 inflammasome: mechanism of activation

Caspase-1 was initially identified as the protease responsible for the maturation and release of IL-1β in response to ATP. Deeper understanding of this process was provided by the discovery that NLRP3 and ASC are required for the activation of caspase-1 in response to ATP and certain bacterial pore-forming toxins[3]. Consistent with the importance of NLRP3 in IL-1β production, *NLRP3* gain-of-function mutations lead to cryopyrin-associated periodic syndromes, which are efficiently treated with inhibitors of IL-1β-mediated signaling[3]. The NLRP3 inflammasome is activated by a plethora of microbial stimuli, including MDP[40,41], bacterial RNA[42], the double-stranded RNA analog poly(I:C)[42], lipopolysaccharide, microbial lipopeptide, the imiquimod R-837 and the synthetic imidazoquinoline resiquimod (R-848)[42]. NLRP3 can also be activated by endogenous stimuli and particulate matter, such as uric acid, cholesterol and hydroxyapatite crystals, silica, aluminum salts, asbestos, malarial hemozoin, amyloid deposits and fatty acids[4]. Given the chemical and structural diversity of the NLRP3 activators, it has been hypothesized that NLRP3 does not interact directly with its activators; instead, its activation is triggered through an intermediate cellular signal elicited by all these stimuli. Evidence indicates that most, if not all, Toll-like receptor (TLR) agonists and MDP do not directly activate the NLRP3 inflammasome. Instead, these microbial stimuli prime the activation of NLRP3 through the induction of NLRP3 expression in macrophages[43], a prerequisite for inflammasome activation[43–45]. Consistent with that, activation of the NLRP3 inflammasome by ATP, bacterial pore-forming toxins and particulate matter requires prestimulation with TLR agonists to induce NLRP3 expression[43–46]. Whereas the adaptor TRIF has a minor role in the priming process in response to stimulation with lipopolysaccharide[47], it has a major role in response to bacterial RNA[48]. Because NLRP3 induction is mediated by the transcription factor NF-κB, endogenous cytokines such as tumor-necrosis factor and IL-1β are also effective in inducing NLRP3 expression and promoting caspase-1 activation in response to NLRP3 activators[45]. Thus, activation of the NLRP3 inflammasome requires two signals in mouse macrophages. The first signal is provided by microbial or endogenous molecules that activate NF-κB and induce NLRP3 expression (**Fig. 2**). The second signal directly activates NLRP3 and is provided by ATP, certain bacterial toxins or particulate matter (**Fig. 2**). The situation may be different in human monocytes and microglia cells, in which stimulation with TLR ligands induces the release of IL-1β without exogenous stimulation with ATP[49–51]. However, stimulation of human monocytes and microglia cells with TLR has been proposed to induce the release of endogenous ATP that acts in an autocrine way to activate the ion channel $P2X_7$ (refs. 49–51). Notably, under conditions in which the autophagic pathway is compromised, stimulation with pathogen-associated molecular patterns induces activation of the NLRP3 inflammasome[52] and more production of pro-IL-1β[53,54], which suggests that autophagy has a role in controlling the production of IL-1β.

Several theories have been proposed for the identity of the cellular signal responsible for NLRP3 activation, including a change in the intracellular concentration of K^+ and Na^+, the formation of a large

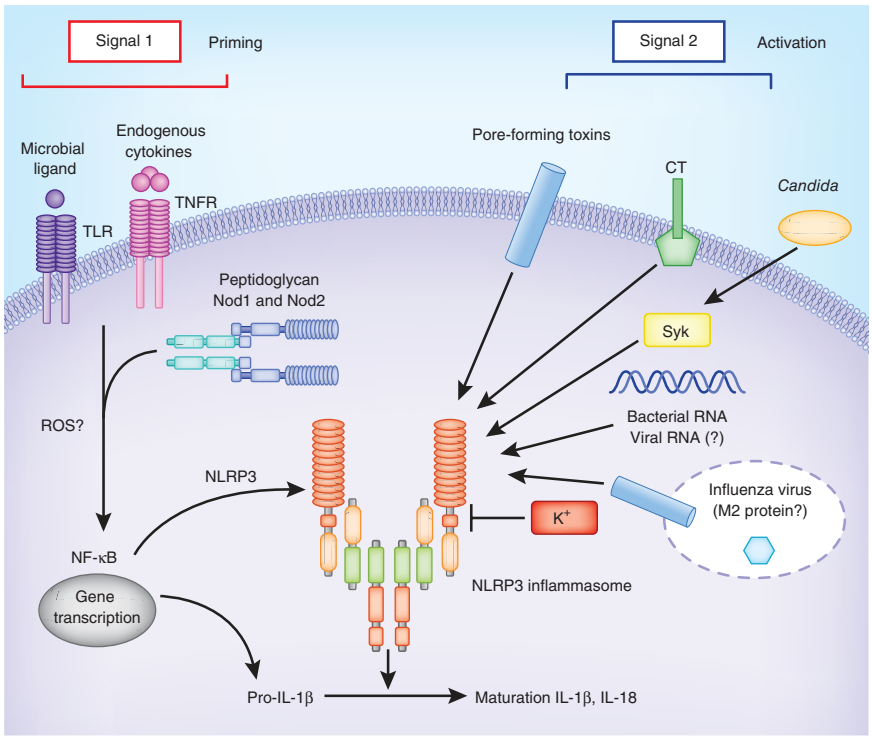

Figure 2 The NLRP3 inflammasome. Activation of caspase-1 via NLRP3 requires two signals. Signal 1 is represented by microbial molecules or endogenous cytokines and is required for the upregulation of NLRP3 and pro-IL-1β. Signal 2 activates the NLRP3 inflammasome. Activation by *S. aureus*, *S. pyogenes*, *S. pneumoniae* and *V. cholerae* is mediated by pore-forming toxins. Other bacterial toxins can also induce the activation of the NLRP3 inflammasome, such as cholera toxin (CT) or *C. difficile* toxins TcdA and TcdB (not shown here); *C. albicans* induces activation of the NLRP3 inflammasome through the kinase Syk, although the mechanism involved is unclear. Influenza virus can induce the activation of the NLRP3 inflammasome, but it is controversial whether this is due to a pore-forming activity mediated by M protein or to sensing of viral RNA species in the cytosol. Cytosolic bacterial RNA induces activation of the NLRP3 inflammasome. TNFR, receptor for tumor-necrosis factor.

pore in cell membranes, the release of cathepsins from damaged lysosomes, the production of reactive oxygen species (ROS) and damage in the mitochondria[3]. The involvement of K^+ efflux in NLRP3 activation is supported by the fact that some NLRP3 activators, including ATP, the antibiotic nigericin and pore-forming toxins, results in lower intracellular concentration of K^+, and a high extracellular concentration of K^+ inhibits activation of the NLRP3 inflammasome[55,56]. However, there is no evidence that particulate matter triggers efflux of K^+. Furthermore, the interpretation of experiments in which extracellular Na^+ is replaced with K^+ is complicated because extracellular Na^+ is also reported to be required for NLRP3 activation independently of K^+ efflux[57]. Hence, it is difficult to discern whether the inhibitory effect of isotonic medium with a high concentration of K^+ on NLRP3 activation is due to the high concentration of K^+ or the low concentration of Na^+. Very high extracellular concentrations of K^+ also block the activation of the NLRP1, NLRC4 and AIM2 inflammasomes[58]. Therefore, further studies are required to clarify the role of changes in cytosolic ionic concentrations in the activation of the NLRP3 inflammasome.

Extracellular ATP activates NLRP3 through the opening of ATP-gated $P2X_7$. In contrast, bacterial pore-forming toxins activate NLRP3 independently of $P2X_7$ (refs. 44,59). $P2X_7$ is unique among ion channels in that its activation not only opens a cation channel but also leads to the opening of a larger pore permeable to molecules 900 Da or greater in size. It has been suggested that the opening of a large pore formed by the hemichannel pannexin-1 after $P2X_7$ stimulation is necessary for NLRP3 activation independently of K^+ efflux[60]. However, no defect in NLRP3 activation or opening of the large pore in pannexin-1-deficient macrophages stimulated with ATP and nigericin has been found[61]. Although those results suggest that pannexin-1 is not the molecular component of the large pore opened by ATP, it is still unknown whether the opening of a large pore is required to activate NLRP3.

The activation of NLRP3 by particulate matter requires endocytosis, as pretreatment of macrophages with drugs that interfere with cytoskeletal dynamics (such as colchicine and cytochalasin B) inhibit the activation of NLRP3 by uric acid crystals, silica and aluminum salts but not by ATP[4]. Furthermore, inhibitors of cathepsin B can prevent the activation of caspase-1 induced by certain microbes[62,63]. However, as cathepsin B–deficient mice have a modest or no defect in the activation pf NLRP3 by particulate matter[64], the observed impairment of NLRP3 activation by the inhibitor of cathepsin B could be due to off-target effects. Alternatively, given the considerable redundancy among the members of the cathepsin family, several lysosomal proteases may be able to trigger NLRP3 activation. Studies of mice with genetic double deficiency could help clarify the role of cathepsins in NLRP3 activation and the mechanism involved.

The production of ROS has also been suggested to act as a common cellular signal upstream of NLRP3 triggered by ATP and particulate matter[65]. NLRP3 activation is blocked by ROS scavengers and NAPDH-oxidase inhibitors[65]. In line with those findings, thioredoxin-interaction protein has been proposed to bind and activate NLRP3 after the production of ROS by NLRP3 activators[66]. However, those results were not independently confirmed in a different study[67]. Furthermore, another report has suggested that ROS inhibitors interfere with NLRP3 priming rather than its activation[46]. ROS derived from the mitochondria have been suggested to mediate activation of the NLRP3 inflammasome in a study using inhibitors of the respiratory chain[68]. However, studies with chemical inhibitors, especially at high concentrations, are prone to artifacts. The release of mitochondrial DNA induced by activators of the NLRP3 inflammasome further amplifies caspase-1 activation[69]. Although none of the aforementioned results provides a satisfactory explanation for the mechanism of NLRP3 activation, a combination of several cellular signals could be required for NLRP3 activation. Indeed, NLRP3 has been proposed to integrate signals that indicate cellular damage or stress, including membrane permeation, lysosomal damage, ROS production and mitochondrial damage.

NLRP3 activation by microbes

Many bacterial pathogens activate the NLRP3 inflammasome through the secretion of pore-forming toxins. *Staphylococcus aureus* α-hemolysin activates the NLRP3 inflammasome in combination with TLR2 stimulation by bacterial lipopeptides released during growth[59].

Analysis of isogenic single-, double- and triple-mutant *S. aureus* strains defective in α-, β- and γ-hemolysins has shown that these have a redundant role in NLRP3 activation[47,59]. *In vivo* experiments with an *S. aureus* subcutaneous abscess model have shown a critical role for ASC and IL-1β signaling in neutrophil recruitment and control of the infection[70,71]. Similar to the redundant role of *S. aureus* hemolysins in NLRP3 activation, *Vibrio cholerae* secrete the hemolysins HlyA and MARTX to activate NLRP3 (ref. 72). Furthermore, *in vivo* studies of mouse strains deficient in inflammasome components have shown that caspase-1 and ASC have a protective role against *V. cholerae*, but NLRP3 does not[72]. These results suggest that multiple inflammasomes contribute to host defense against *V. cholerae in vivo*.

Streptococcus pneumoniae, which colonizes the upper respiratory tract, is a leading cause of pneumonia and meningitis and activates NLRP3 through the secreted pore-forming toxin pneumolysin[73,74]. In an *S. pneumoniae* lung-infection model[73,74], NLRP3 elicits a protective immune response, as *Nlrp3*$^{-/-}$ mice have higher bacterial loads and greater mortality than do wild-type mice. In contrast, *Nlrp3*$^{-/-}$ and ASC-deficient (*Pycard*$^{-/-}$) mice develop less brain inflammation and have a better clinical outcome than that of wild-type mice in a pneumococcal meningitis model[75]. Blockade of caspase-1-mediated signaling with a combined regimen of recombinant IL-1 receptor antagonist and IL-18-binding protein leads to considerable amelioration of disease severity and brain pathology, which suggests that interfering with inflammasome activation might be a strategy for treating pneumococcal meningitis.

Several other bacterial toxins induce activation of the NLRP3 inflammasome, including cholera toxin B, adenylcyclase toxin[76] and *Clostridium difficile* toxins A and B[77]. As these toxins have different mechanisms of action, it remains unclear how they mediate activation of the NLRP3 inflammasome. Such activation mediated by cholera toxin B depends on caspase-11, but activation mediated by adenylcyclase toxin, *C. difficile* toxin B or pore-forming toxins does not[76]. Notably, caspase-11 is dispensable for the activation of caspase-1 by most stimuli that activate the NLRP3 inflammasome but has a role in the induction of pyroptosis and release of damage-associated molecular patterns[76]. Some T3SS effector proteins induce activation of the NLRP3 inflammasome. For example, activation by the *Yersinia pestis* subspecies Kim has been described for the effector protein YopJ[78], an acetyltransferase that causes apoptosis through inactivation of kinases such as MAPKs and IKKs[79]. The role of NLRP3 in *Y. pestis* infection *in vivo*, however, remains to be determined.

Several studies have reported a role for NLRP3 in the innate immune response to viruses. Initial studies showed that the NLRP3 inflammasome can be activated *in vitro* by Sendai virus[80], influenza A virus[80] and adenovirus[81]. Influenza A virus activates NLRP3 through the proton-selective M2 channel[82] and elicits a protective inflammatory response[83–85]. However, there is conflicting evidence about the contribution of NLRP3 to the control of viral burden, host survival and the generation of adaptive immunity after influenza infection[83–85]. In an initial analysis[83], mice deficient in ASC, caspase-1 or the IL-1 receptor had more mortality accompanied by lower immunoglobulin responses than wild-type mice had after infection with influenza virus, but those deficient in NLRP3 did not. However, in two subsequent studies[84,85], *Nlrp3*$^{-/-}$, *Pycard*$^{-/-}$ and *Casp1*$^{-/-}$ mice had more mortality than wild-type mice but no defect in the generation of adaptive immunity to influenza[84]. The reason for these contradictory results is unclear.

Candida albicans, a fungus that can cause severe opportunistic infections in immunocompromised hosts, can activate the NLRP3 inflammasome[86]. Experiments with *C. albicans* at various morphological stages[87,88] and mutants that cannot form hyphae have shown that the yeast form is a more potent activator of NLRP3 than is the hyphal form; furthermore, the transition from yeast to hyphal form is an important step in eliciting NLRP3 activation[87]. Responsiveness to *C. albicans* requires TLR2, the receptor for dectin-1, and the signaling molecule Syk and its downstream adaptor CARD9 for the priming step, whereas Syk is required for activation of the NLRP3 inflammasome but CARD9 is not[86,88]. *In vivo* experiments with *Tlr2*$^{-/-}$, dectin-1-deficient, *Nlrp3*$^{-/-}$, *Pycard*$^{-/-}$, *Casp1*$^{-/-}$ and IL-1 receptor–deficient mice have demonstrated a protective role for the NLRP3 inflammasome in a model of disseminated candidiasis[88]. Notably, both NLRP3 and NLRC4 are required for host defense in a model of oral candidiasis[89]. However, the mechanism by which *C. albicans* activates the NLRC4 inflammasome is unclear.

Malaria is caused by *Plasmodium* parasites, which feed on erythrocyte hemoglobin and use a heme-detoxification mechanism that leads to the formation of dark-brown crystals called 'hemozoin'. As with other particulate matter, there is evidence that hemozoin crystals activate the NLRP3 inflammasome after phagocytosis[64]. Whereas one study found a modest but important role for NLRP3 in promoting cerebral malaria[90], subsequent studies have found no evidence for contribution of NLRP3, ASC, caspase-1, IL-1β or IL-18 in the development of cerebral malaria[91], so the role of the NLRP3 inflammasome in this disease remains controversial.

Redundancy in inflammasome activation

Bacterial infection can trigger the activation of several inflammasomes. The clearest example is infection by the intracellular pathogen *Listeria monocytogenes*. Initial studies suggested that *L. monocytogenes* induces activation of the NLRP3 inflammasome, whereas other studies have found NLRP3 to be dispensable for the activation of caspase-1[92]. That apparent discrepancy has been reconciled by the observation that *L. monocytogenes* can engage multiple inflammasomes and that the contribution of each inflammasome (NLRP3, NLRC4 or AIM2) depends in part on the experimental conditions of infection[93–96]. Although these results are controversial, it has been reported that *Casp1*$^{-/-}$ and *Pycard*$^{-/-}$ mice are more susceptible than wild-type mice are to *L. monocytogenes* infection[27,97,98]. However, it is unclear whether a particular inflammasome is dominant or whether multiple inflammasomes are redundantly activated *in vivo*. As with *L. monocytogenes*, several inflammasomes can be activated, depending on the experimental conditions, by other bacteria, including *S. enterica*[5,6,24] and *S. flexneri*[13,14,99], as well as by fungi[89] (**Table 1**). However, except for *S. enterica*, for these pathogens, the specific contribution of each inflammasome to host defense *in vivo* remains largely unknown. Mice deficient in both NLRC4 and NLRP3 (*Nlrc4*$^{-/-}$*Nlrp3*$^{-/-}$ mice) are slightly more susceptible to infection with *S. enterica* than wild-type mice are, but those deficient in either inflammasome alone are not; this is correlated with a five- to ten-fold greater pathogen burden[24]. Consistently, the phenotype of *Nlrc4*$^{-/-}$*Nlrp3*$^{-/-}$ mice is similar to that of *Casp1*$^{-/-}$ mice[23,24]. The role of ASC in host defense against infection with *S. enterica* is more complex. ASC comprises a pyrin domain and a CARD and is thought to be an essential adaptor that connects NLRP3 to caspase-1 (ref. 3). Experiments with mice deficient in ASC have shown that ASC is necessary for the activation of caspase-1 and the maturation of IL-1β after infection of mice with *S. enterica*, *P. aeruginosa* or *L. pneumophila*[92]. Notably, ASC is dispensable for the induction of pyroptosis[6,11,38], which in these infection models depends on caspase-1 but not on caspase-11 (ref. 38). Studies have shown that induction of pyroptosis does not require the proteolytic maturation of caspase-1 and suggest that phagocytes can assemble two different inflammasomes in response to infection with

Table 1 Activation of inflammasomes by microbes

Pathogen	Microbial activator	Inflammasome	References
Bacterial			
Streptococcus pneumoniae	Pneumolysin	NLRP3	73,74
Klebsiella pneumonia	Unknown	NLRP3	101
Bordetella pertussis	CyaA	NLRP3	102
Clostridium difficile	TcdA and TcdB	NLRP3	77
		(?)	76
Yersinia species (*Y. pestis* KIM subspecies and *Y. pseudotuberculosis*)	YopJ	NLRP3, NLRC4	78,79
Staphylococcus aureus	Hemolysins	NLRP3	59
Vibrio cholerae	HlyA and $MARTX_{Vc}$	NLRP3	72
	Cholera toxin B		76
Escherichia coli	RNA	NLRP3 or TRIF	48
	Heat-labile enterotoxin (lethal toxin)	NLRP3	103
Citrobacter rodentium	Unknown	NLRP3	76
Streptococcus pyogenes	Streptolysin O	NLRP3	44
Chlamydia pneumonia	Unknown	NLRP3	104,105
Neisseria gonorrhea	Unknown	NLRP3	62
Mycobacterium tuberculosis	Unknown	NLRP3, NLRC4	106–108
Listeria monocytogenes	LLO, flagellin, bacterial DNA	NLRP3, NLRC4, AIM2	55,94,109–112
			27
Aeromonas hydrophila	Aerolysin	NLRP3, NLRC4	113
Salmonella typhimurium	Flagellin,	NLRC4	5,6,14
	PrgJ	NLRP3	24
Shigella flexneri	MxiI?	NLRC4	13,14
	?	NLRP3	99
Pseudomonas aeruginosa	Flagellin	NLRC4	10,11,15,114
Legionella pneumophila	Flagellin	NLRC4 (Naip5)	7–9,16,17,115,116
Bacillus anthracis	Lethal toxin	NLRPlrp1Bb	37,39,117
Franciscella tularensis	Bacterial DNA	AIM2	38,93,118
Fungal			
Candida albicans	Unknown	NLRC4, NLRP3	86–89
Aspergillus fumigatus	Unknown	NLRP3	119
Viral			
Sendai virus	Unknown	NLRP3	80
Influenza A	Viral M2, viral RNA?	NLRP3	82,84,85
Adenovirus	Unknown	NLRP3	81
Varicella zoster	Unknown	NLRP3	120
Cytomegalovirus	Viral double-stranded DNA	AIM2	93
Vaccinia virus	Viral double-stranded DNA	AIM2	93,121
Herpes virus	Unknown	NLRP3	120

S. enterica[38]. One inflammasome containing NLRC4 and caspase-1 is responsible for the induction of pyroptosis, whereas the other inflammasome, containing NLRC4, ASC and caspase-1, mediates the maturation of IL-1β and IL-18 (ref. 38). These data suggest that microbial infections can activate different NLRC4-containing inflammasomes that exert different functions. More detailed analysis of the composition and biochemical properties of different protein complexes in the NLRC4 inflammasome is needed to determine the relevance of these findings.

Pathogen-specific recognition by the inflammasome

Commensal microorganisms abundant in the skin and intestines continuously challenge the immune system without eliciting an inflammatory response. TLRs detect microbial ligands present in the extracellular environment and are activated by both commensal and pathogenic bacteria. However, the keratinized epithelium of the skin and the mucus layer of the gut form a physical barrier that prevents noninvasive microbes from engaging TLRs. In contrast to TLRs, NLR proteins sense the presence of microbial ligands in the cytosol. Thus, members of this class of PRRs are ideal sensors of pathogenic bacteria because bacterial secretion systems or pore-forming toxins, which are features of pathogenic bacteria, can promote the delivery of microbial ligands to the host cytosol. The presence of bacterial secretion systems and pore-forming toxins is important for activation of the inflammasome and production of IL-1β. For example, activation of the NLRC4 inflammasome by pathogenic bacteria requires a functional T3SS or T4SS[92]. Similarly, activation of the NLRP3 inflammasome by *S. aureus*, *V. cholerae* and *Streptococcus pyogenes* requires bacterial pore-forming toxins[4,92]. In contrast to peripheral tissues, the intestine is populated with specialized resident phagocytes that are hyporesponsive to microbial stimulation[100]. It will be important to determine whether the inflammasomes have a role in the discrimination of pathogenic versus nonpathogenic bacteria in the gut.

Conclusions and future perspectives

In the past decade, much progress has been made in elucidating the activation, regulation and function of the inflammasomes in response to microbes. Researchers have identified the microbial sensors responsible for the activation of caspase-1 and a role for Naip proteins in promoting NLRC4 activation. Moreover, there is conclusive evidence that the inflammasomes contribute to host defense against a variety of pathogens. However, the molecular mechanism by which Naip proteins activate NLRC4 and different stimuli induce activation of the NLRP3 inflammasome remains largely unknown. Furthermore, little is known about the protein substrates cleaved by caspase-1 and/or caspase-11 and their role in executing pyroptosis. Another unresolved question is how inflammasomes and other signaling pathways act together *in vivo* to orchestrate innate and adaptive immune responses. Clearly, much remains to be learned about the inflammasomes and their role in microbial recognition and host defense against microbes.

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